

## Health and Performance of Holstein Bull Calves fed *Aspergillus Oryzae* Fermentation Extract

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### ABSTRACT

The objective of this study was to determine whether feeding Amaferm, a fermentation extract of *Aspergillus oryzae*, improved the health and performance of Holstein bull calves from birth to weaning. Calves were randomly assigned to a slaughter age (4 wk (n = 16) or 8 wk (n = 36)) and treatment (control (CON; n = 27) or Amaferm (AMF; n = 25)). AMF calves were fed 2 g of AMF daily, delivered in milk replacer for the first 4 wk of life and top-dressed on starter thereafter. Calves were fed milk replacer twice daily and were weaned upon consuming 0.91 kg of grain daily or on d 45 of the study. Calves had ad libitum access to grain and water. Milk replacer and starter intakes were recorded daily. Body weight was recorded weekly. Fecal scores were assigned twice daily. Upon slaughter, jejunal lymph nodes were collected for flow cytometric analysis of CD4 and CD8 cell populations. Body weights and medical costs were consistent between treatments. Calves fed AMF had greater frequency of scours than calves not fed AMF. CD4 cells composed a higher percentage of observed cells in AMF calves, while there was no difference in CD8 cell percentages between treatments. In conclusion, calves fed AMF scoured more frequently, but a lesser percentage of AMF calves were treated for respiratory ailments leading to no effect on treatment costs. Interestingly, CD4 cell population was greater in AMF calves, which warrants further research.

### INTRODUCTION, JUSTIFICATION, AND HYPOTHESIS

The period from birth to weaning is a stressful time for dairy calves. The most prevalent causes of death from during this period are scours and respiratory problems (USDA NAHMS 2011). Calf mortality and treatment costs represent an enormous economic loss to the dairy industry, estimated to be greater than \$250 million annually (Davis and Drackley, 1998).

Amaferm (AMF) (Biozyme, Inc.; St. Joseph, MO), a fermentation extract of *Aspergillus oryzae*, is a direct fed microbial with probiotic and prebiotic properties. AMF has been studied in both ruminants and nonruminants with favorable outcomes in fiber digestibility (Chang et al., 1999) and suspected benefits in gut health (<http://www.biozymeinc.com/research/pigs.html>), respectively. AMF may set calves up for better feed efficiencies and possibly favorable immune function and health.

To ascertain if AMF is efficacious at improving growth and health in preweaned calves, a study must be done with AMF included in calf milk replacer and calf starter. Calves must be weighed weekly to answer questions about weight gain. Calf health must be monitored in order to detect improvement in immune function. Lastly, flow cytometry must be performed on intestinal lymph nodes to examine CD4 and CD8 cell populations in order to detect differences in T cell population as a measure of immune function. The study outlined below aimed to do all of these things.

It was hypothesized that calves fed 2 g of AMF daily, delivered in calf milk replacer for the first 4 wk of life and top-dressed on starter thereafter, will have lower fecal scores and treatment costs. Further, it was hypothesized that CD4 and CD8 cell populations of intestinal lymph nodes will differ between treatments.

### MATERIALS AND METHODS

#### Calves, Diets, and Management:

The Ohio State University Animal Care and Use Committee approved all animal procedures (protocol # 2013A00000059). In this 8 wk trial, 52 locally sourced (Wayne County, OH) Holstein bull calves were transported to the OARDC Small Ruminant Research Center

(Wooster, OH) on May 6<sup>th</sup>, 2013. Calves were blocked by source farm (n = 14), age (0 to 6 d of age), initial body weight, initial IgG score (moderate or high), and lactation number of dam (primiparous or multiparous) and randomly assigned to a slaughter age (4 wk (n = 16) or 8 wk (n = 36)), and treatment (control (**CON**; n = 27) or Amaferm (**AMF**; n = 25)).

Calves were individually housed in a naturally ventilated barn; the experimental unit was calf. Pens had no bedding. Calves were fed non-medicated milk replacer (**MR**; 22% CP, 20% fat, DM basis) from buckets twice daily at 0600 and 1800h. Calves were offered 0.68 kg of MR powder mixed in 3.78 L of water per day; MR was delivered in 2 equal portions. Calves assigned to AMF received 2g/d of liquid AMF product, delivered in equal portions of MR for the first 4 wk of the trial. Calves had freechoice access to a medicated (0.0033% Decoquinat) 20% CP (DM basis) calf starter and water at all times. For the final 4 wk of the trial, AMF was top-dressed on calf starter (2 g/d; offered at 0600h feeding). Calves began the 5-d weaning process when they consumed 0.91kg of starter for 3 consecutive days or on day 45 of the trial, whichever came first. During the weaning process, MR amount was reduced by 50% and was offered at the 0600h feeding only. Calves were not dehorned or castrated. Calf starter and MR intake were measured daily.

### **Growth and Health Monitoring**

Calves were weighed weekly. All vaccination procedures and veterinary care were provided by the attending OARDC veterinarian. Upon arrival all calves received 1 mL/nostril Inforce-3 (Zoetis, Florham Park, NJ) and 2 mL Excenel (Zoetis) subcutaneously. On d 5 all calves received 2 mL Ultrachoice 7 (Zoetis) subcutaneously. Calves received 2 mL preventative Excenel subcutaneously on days 4, 6, 7 and 8. In addition, 1 mL per nostril Inforce 3 was administered on d 8. Calves being slaughtered at 8 wk received 2 mL Ultrachoice 7 subcutaneously at d 24. This was not administered to 4 wk calves due to withdrawal time. Calves did not receive injections of selenium and vitamins A, D, and E due to the 60 d withdrawal period of these injections.

A 4-point scale was used for twice daily fecal scoring (Diaz et al., 2001). Any calf with a fecal score of 3 or greater was offered 1.89 L oral electrolytes (Land O' Lakes Inc., Shoreview, MN). Prevail (Vetone, MWI Veterinary Supply, Boise, ID) was used to treat fever (rectal temperature  $\geq 39.4$  °C) and discomfort. Infections were treated under the instruction of the attending OARDC veterinarian primarily with injectables: Excenel, Draxxin (Zoetis), Nuflor (Merck Animal Health, Summit, NJ), or Sustain III calf boluses (Bimeda Inc., Oakbrook Terrace, IL). When determining treatment for a symptomatic calf, care was taken to select a regimen that did not violate pre-slaughter withdrawal periods. All treatments were recorded for calculation of treatment costs (**Table 1**).

**Table 1. Cost per mL of medications (MWI Veterinary Supply, Boise, ID).**

Drug	Cost/mL
Inforce-3	\$ 0.55
Ultrachoice 7	\$ 0.26
Excenel	\$ 0.73
Prevail	\$ 0.10
Draxxin	\$ 3.90
Nuflor	\$ 0.62
Sustain III	\$ 0.79

## Sample Collection and Flow Cytometric Analysis

For slaughter, animals were transported approximately 90 miles to the meat science laboratory at The Ohio State University. Feed was not withheld prior to slaughter. Calves were slaughtered by captive-bolt stunning followed by exsanguination. Intestinal lymph nodes (~1 g/calf) were collected from the mesentery surrounding the jejunum and transported back to Wooster stored at 4°C in RPMI (Sigma-Aldrich, St. Louis, MO).

Each lymph node sample was pressed through a 40 µm cell strainer. The effluent was added to 15 mL ice cold RPMI. The lymph cells were centrifuged at 1800 rpm for 5 min at 10°C. Cells were washed once with RPMI and resuspended using running buffer (5 mL 10x PBS, 200 µL 0.5 M EDTA, 1.5 mL normal goat serum, 43.3 mL autoclaved H<sub>2</sub>O). The cells were stained with FITC-conjugated mouse anti-bovine CD8 monoclonal antibodies (AbD Serotec, Raleigh, NC) and RPE-conjugated mouse anti-bovine CD4 monoclonal antibodies (AbD Serotec). Cells were incubated for 15 min at 4°C. After washing thrice, cells were resuspended in running buffer and analyzed on a flow cytometer (EMD Millipore Corp, Billerica, MA).

## Data Analysis

Data were analyzed using the Proc Mixed Procedure of SAS (version 9.2; SAS Institute; Cary, NC). Calf within treatment was the random term. Fixed effects in the model included treatment, kill date, and their interaction. The Proc Frequency Procedure of SAS was used to generate frequency distribution of categorical data (fecal score and respiratory treatments). Data were declared significant when  $P \leq 0.05$ .

## RESULTS

Body weight of 8wk slaughter calves throughout the study was not affected by treatment (data not shown;  $P=0.832$ ). On a weekly basis, calves fed AMF exhibited scours (fecal score >2) more frequently than CON calves (**Figure 1**;  $P=0.01$ ). Scours were more frequent in all calves, regardless of treatment, in the early weeks of the trial (**Figure 1**;  $P=0.01$ ). Treatment for respiratory ailments were more frequent in CON calves than in calves fed AMF (**Figure 2**;  $P=0.01$ ).

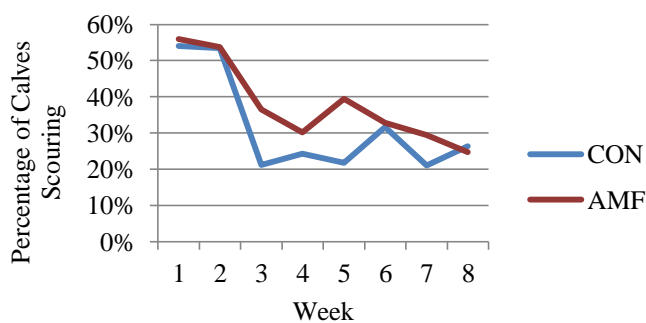


Figure 1. Percentage of calves scouring over time.

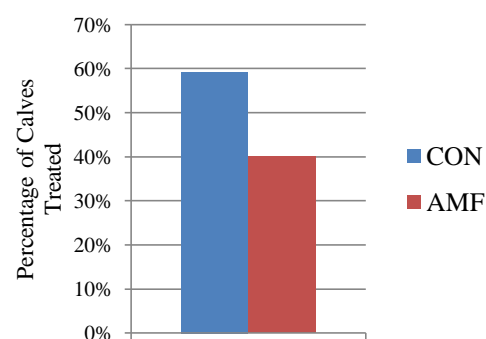


Figure 2. Percentage of calves treated for respiratory ailments.

The average total treatment cost per calf (**Table 2**) includes the cost of all vaccinations, medications and electrolytes. Treatment costs were not different between calves on AMF or CON in either wk 1-4 or wk 5-8 ( $P=0.594$  and  $0.306$ , respectively).

Table 2. Average total treatment cost per calf

	wk 1-4 (n=52)	wk 5-8 (n=36)
CON	\$ 42.79 +/- \$2.33	\$ 9.46 +/- \$2.33
AMF	\$ 43.24 +/- \$ 2.47	\$ 12.90 +/- \$2.47

The CD4 cell population of intestinal lymph nodes as a percentage of total observed cells was greater in AMF calves (**Table 3**;  $P=0.050$ ). Treatment did not affect CD8 cell population as a percentage of total observed cells (**Table 3**;  $P=0.427$ ).

**Table 3. CD4 and CD8 as a Percentage of Observed Cell Population**

	CD4	CD8
CON	$24.44 \pm 1.65\%$	$5.49 \pm 0.61\%$
AMF	$29.78 \pm 1.74\%$	$7.45 \pm 0.85\%$

## DISCUSSION

Scours and respiratory disease are the two greatest causes of calf morbidity and mortality in the US (USDA NAHMS 2011). Two calves on the AMF treatment died due to scours related causes. Data from these animals are not reported and these animals were not replaced. In 2010, the national mortality rate of preweaned heifers on heifer raising operations was 4.2% (USDA NAHMS 2011). The mortality rate for this trial was 3.7%, which is below the national average. 16.4 % of heifers on heifer raising operations are treated for pneumonia (USDA NAHMS 2011), while 50% of calves on this trial were treated for unspecified respiratory ailments. No calves in this trial were treated with antibiotics because of scours, while the national average for antibiotic scours treatment is 18.2 % (USDA NAHMS 2011).

It is common for calves to scour within the first 4 wk of life (USDA NAHMS 2011), and this was observed in this study. We originally hypothesized that AMF would lower fecal scores. This is not what we found. However, a lesser percentage of AMF calves were treated for respiratory ailments. This was unexpected, given that AMF is a direct fed microbial and reasons behind this difference should be explored in future studies. Given that AMF calves were treated more for scours but less for respiratory, the overall treatment cost did not differ between treatment, though the cost for AMF calves was numerically higher in both the wk 1-4 and wk 5-8 periods.

CD4 cell percentages were higher in AMF calves than in CON calves. This leads us to hypothesize that calves fed AMF would be more prepared to fight infection and therefore healthier. Future analyses in our lab from these calves will evaluate relative abundance of pro- versus anti-inflammatory cytokines in order to reveal whether the CD4 cells present were actively fighting an infection (increased pro-inflammatory) or merely regulatory (increased anti-inflammatory). It should be noted that cell populations could only be recorded at the time of slaughter, which does not indicate health status over time. Further analysis is necessary to determine the implications and reasons for these higher CD4 populations. While differences in CD4 populations were observed in 8 wk old calves due to treatment, CD8 cell populations were unaffected. CD8 is more associated with innate immunity than adaptive immunity. Taking together our results on CD4 and CD8 cell populations indicate that AMF may affect the adaptive immune system through effects mediated by CD4 positive cells. More research is needed in this area.

In conclusion, calves fed AMF scoured more frequently, but a lesser percentage of AMF calves were treated for respiratory ailments leading to no effect on treatment costs. Interestingly, the CD4 cell population was greater in AMF calves, which warrants further research.

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